

Clutch size and malarial parasites in female great tits

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Life-history models predict an evolutionary trade-off in the allocation of resources to current versus future reproduction. This corresponds, at the physiological level, to a trade-off in the allocation of resources to current reproduction or to the immune system, which will enhance survival and therefore future reproduction. For clutch size, life-history models predict a positive correlation between current investment in eggs and the subsequent parasite load. In a population of great tits, we analyzed the correlation between natural clutch size of females and the subsequent prevalence of *Plasmodium* spp., a potentially harmful blood parasite. Females that showed, 14 days after hatching of the nestlings, an infection with *Plasmodium* had a significantly larger clutch ($9.3 \text{ eggs} \pm 0.5 \text{ SE}$, $n = 18$) than uninfected females ($8.0 \text{ eggs} \pm 0.2 \text{ SE}$, $n = 80$), as predicted by the allocation trade-off. Clutch size was positively correlated with the prevalence of *Plasmodium*, but brood size 14 days after hatching was not. This suggests that females incur higher costs during laying the clutch than during rearing nestlings. Infection status of some females changed between years, and these changes were significantly correlated with a change in clutch size as predicted by the trade-off. The link between reproductive effort and parasitism may represent a possible mechanism by which the cost of egg production is mediated into future survival and may thereby be an important selective force in the shaping of clutch size.

Key words: blood parasites, clutch size, *Parus major*, *Plasmodium* spp., trade-off. [*Behav Ecol* 8:148–152 (1997)]

A trade-off in the allocation of resources to reproduction or to the immune system predicts that an increase in reproductive effort will be associated with an increase in the prevalence of parasites. Two recent studies on great tits manipulated brood size and indeed found that the prevalence of blood parasites in males caring for enlarged broods is significantly higher than in males caring for unmanipulated and reduced broods (Norris et al., 1994; Richner et al., 1995). Males that were given two extra young increased their rate of food provisioning to the nest by 50% (Richner et al., 1995), and also showed an increase in the prevalence of *Plasmodium* from 35% to 76%. There was no effect of brood size manipulation on prevalence of blood parasites in females. One of the studies (Richner et al., 1995) showed that females do not respond to the experimental enlargement of the brood by an increase in their rate of food provisioning to the nest, thus explaining why parasite prevalence did not increase in females. In both of these studies, brood size was manipulated after the egg-laying phase, and so these studies cannot address the question of a trade-off between egg production and parasitism. Under the trade-off hypothesis, we predict a positive correlation between natural clutch size and the prevalence of blood parasites. Alternatively, under the "individual optimization hypothesis" (e.g., Pettifor et al., 1988), the clutch size of each female should match her individual investment capacity, and a correlation between natural clutch size and the prevalence of blood parasites would therefore not be predicted.

Blood parasites of the plasmodium type are frequently observed in the great tits living in our study area, and they are common in many bird species (Seed and Manwell, 1977). Their pathogenic effects include hemolytic anemia, a lower metabolic rate, poorer thermoregulation (Hayworth et al., 1987), and reduced survival and fecundity (Atkinson and Van Riper, 1991; Van Riper et al., 1986). Infected hosts may de-

velop a chronic or latent infection in which the immune system of the host controls the parasite (Atkinson and Van Riper, 1991). During this phase of infection, the parasites are not found in a blood smear but will reappear under conditions of physiological or environmental stress (Garnham, 1966).

In this nonexperimental study, we investigated (1) the relationship between infection status of females and their age, morphometry and laying date, (2) the relationship between infection status of females and their natural clutch size and brood size 14 days after hatching of the chicks, (3) the relationship between an interannual change in clutch size of individual females and a change in the infection status, and (4) the correlation between infection status, reproductive success, and annual survival of females.

MATERIALS AND METHODS

The study was carried out at two sites around Lausanne, Switzerland, during the breeding seasons of 1992–1994. One site comprised a forest surrounding the university campus, and the other site comprises a forest 10 km west of Lausanne (for further details, see Richner et al., 1993). Starting at the end of March, we visited the nest-boxes regularly to determine laying date, clutch size, and hatching date. Breeding lasts from April to June, and fewer than 10% of the birds that successfully raised a brood lay a second clutch. Adult great tits were caught with a door trap at the nest-box when the nestlings were 14 days old. We measured body mass and tarsus length of both adults and nestlings and classified the parents as yearlings or adults, according to the criteria given by Svensson (1984). We then collected 10 μ l of blood from the brachial vein in a heparinized microcapillary tube. A small droplet of blood was used to produce a thin smear on a glass slide. The smear was air-dried and fixed with absolute methanol and subsequently stained with May-Grünwald Giemsa stain. Each smear was screened for 10 min by light microscopy ($\times 1000$) under oil immersion, and schizont stages of *Plasmodium* were scored as present or absent. Although 140 breeding females were screened, only 2 females were infected by another hematzoic species (*Haemoproteus* spp.). For further verifica-

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Table 1

Mean (\pm SE) laying date, clutch size, body mass, and tarsus length of females infected and uninfected with *Plasmodium*

Variables	Infected	Uninfected	Statistics	<i>p</i>
Laying date (1 = 1 April)	18.9 \pm 1.5 (<i>n</i> = 18)	21.1 \pm 1.1 (<i>n</i> = 80)	<i>t</i> = 0.90	.38
Clutch size	9.33 \pm 0.5 (<i>n</i> = 18)	8.00 \pm 0.2 (<i>n</i> = 80)	<i>t</i> = 2.74	.007
Body mass (g)	16.9 \pm 0.2 (<i>n</i> = 16)	17.1 \pm 0.1 (<i>n</i> = 77)	<i>t</i> = 0.71	.48
Tarsus length (mm)	22.0 \pm 0.2 (<i>n</i> = 15)	22.0 \pm 0.1 (<i>n</i> = 77)	<i>t</i> = 0.037	.97
Age	5 juveniles + 9 adults	29 juveniles + 40 adults	<i>G</i> = 0.194	.66

The number of juvenile and adult females include the birds only that could be aged unambiguously.

tion of blood parasite identification, we sent 20 infected smears to a well-recognized parasitologist (M. Anwar, Oxford University): he identified the parasite in 19 of these smears as *Plasmodium* and found in one smear both *Plasmodium* and *Haemoproteus*. *Plasmodium* is most commonly transmitted by various species of mosquitoes. After infection, birds can have chronic, but latent infections which can relapse. Of 100 juvenile and adult females analyzed for *Plasmodium* in early and late winter by screening of a blood smear, none showed a patent infection. Infection or relapse of a latent infection must therefore arise during the breeding period.

For statistical analysis, we grouped the 140 females according to whether they had been sampled during one breeding season (98 females), or during 2 or 3 seasons (42 females). To avoid pseudoreplication, we used the females recorded during only one season for the main analysis of the relationship between hematozoon infection and laying date, morphometry, female age, and clutch size. The females sampled during more than one breeding season were used for an intraindividual analysis of changes in clutch size in relation to changes in infection status. This analysis is restricted to years 1993 and 1994 in order to use each individual only once in the pairwise comparison, and the year combination of 1993 and 1994 were chosen because they gave a higher sample size

than the year combinations of 92 and 93. Simple statistics (*t* tests, and *G* tests for 2×2 frequency tables) were computed by use of the Systat Statistical Package (Wilkinson, 1989). For analysis of the binary dependent variable presence or absence of *Plasmodium*, a logistic regression model of the type $\ln[p/(1-p)] = a + b^*(\text{clutch size})$ was fitted to the data (Glim 3.77, update 1, Royal Statistical Society, London), where *p* is the binomial probability, and *a* and *b* are the constants generated by the logistic regression model. The significance levels were determined from the change in deviance (denoted as ΔD) which is approximated by a χ^2 distribution with corresponding degrees of freedom (McCullagh and Nelder, 1989).

RESULTS

Infection status of females in relation to laying date, morphometry, and age

Plasmodium was present in 18 of 98 females. There was no difference in mean laying date between infected and parasite-free females (Table 1), nor in body mass or tarsus length. Among the 83 females that could be unambiguously aged (Svensson, 1984), there was no association between female age and infection status (Table 1), and there was also no difference in clutch size between first year (mean \pm SE: 8.3 eggs \pm 0.3, *n* = 34) and adult (8.3 eggs \pm 0.3, *n* = 49) females. Since clutch size was not influenced by female age, a correlation between infection status and clutch size, as shown below, could therefore not have been indirectly caused via an effect of age on clutch size.

Clutch size, brood size, and hemoparasites

Infected females had significantly larger clutches (*t* test: *t* = 2.70; *p* = .007) than noninfected females (Table 1), and the prevalence of *Plasmodium* infection increased with clutch size (Figure 1). We analyzed the relationship between malarial infection and clutch size with a logistic regression model and included year and study site as factors. Clutch size was correlated with infection, as shown by a significant change in deviance from the null model (*p* < .01; Table 2). Including year as a factor led to a further significant change (*p* < .05) in de-

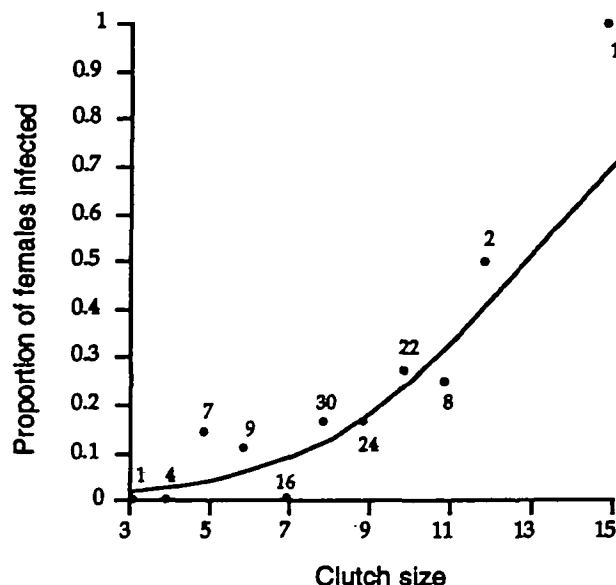


Figure 1. The relationship between clutch size and prevalence of *Plasmodium* in female great tits. The line corresponds to the fitted values of the logistic regression model (see Methods), the points are the observed values. Sample sizes above points correspond to the number of females screened per given clutch size.

Table 2

General linear logistic model for the relationship between infection status of females (dependent variable) and clutch size and year

Model description	df	Deviance (D)	ΔD
Null model	31	37.657	
+Clutch size	30	30.231	7.426**
+Clutch size and year	28	22.003	8.23*

** *p* < .01; * *p* < .02.

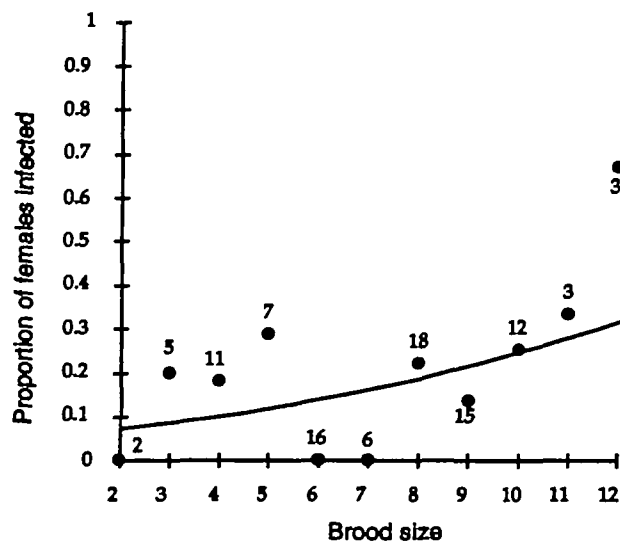


Figure 2
The relationship between brood size 14 days after hatching and prevalence of *Plasmodium* in females great tits. The line corresponds to the fitted values of the logistic regression model (see Methods), the points are the observed values. Sample sizes above points correspond to the number of females screened per given brood size.

viance, but site and all interactions had no further significant effect. Excluding the female with a clutch size of 15 eggs from the analysis had no effect on the above result: clutch size led to a significant change in deviance from the null model ($\Delta D = 5.11$, $p < .01$), and the inclusion of year led to a further significant change ($\Delta D = 7.94$, $p < .05$). The effects of site and all interactions are not statistically significant.

Using the same type of analysis, there was no significant correlation (Figure 2) between *Plasmodium* prevalence in females and brood size 14 days after hatching, even though the demand of food by the nestlings is highest around that period (Van Balen, 1973). Including year, site, and the interactions in the logistic regression model did not significantly change the deviance from the null model.

Hemoparasites and intraindividual changes of clutch size

Infection status of an individual female may also change between years. All possible sequences of infections were found; i.e., infected females not being infected the following year and vice versa, and no change in infection status between years (Table 3).

The females captured in both 1993 and 1994 (Table 3) provided a sufficiently large sample for an intraindividual analysis of infection status in relation to changes of clutch size. Overall, for the 20 females that did not change infection status between the two years (i.e., + + or - - in both years, Table 4) the mean difference in clutch size between the 2 years was $0.35 \text{ eggs} \pm 0.39 \text{ SE}$ (paired t test: $t = 0.89$, $df = 19$, $p = .38$). For the 11 females that changed infection status (i.e., from - to +, or from + to -), clutch size changed significantly in the predicted direction (i.e., an increase if infection status changed from - to +, and a decrease if it changed from + to -) by $1.55 \text{ eggs} \pm 0.54 \text{ SE}$ (paired t test: $t = 2.83$, $df = 10$, $p = .018$; Table 4).

Table 3

Infection status of individual female over several breeding seasons

Infection status			No. of individuals
1992	1993	1994	
-	-	-	4
+	-	+	1
+	+	-	2
-	-	-	11
	-	-	15
	+	+	6
	+	-	2
	+	+	1

(+) Indicates an infection with *Plasmodium*; (-) indicates no infection.

Recapture in relation to infection status

There was no relationship between infection status in 1993 and probability of recapture in 1994 ($G = 1.274$, $df = 1$, $p = .259$). Among the females that were uninfected in 1993, 29% (41 out of 141 females) were recaptured in 1994, and among the infected females 42% (8 of 19) were recaptured in 1994.

Nestling quality, number, and mortality

Nestlings of infected mothers were not lighter or smaller 14 days after hatching than the nestlings of uninfected mothers. Because infected females had larger clutches than uninfected females, and hatching success (number hatched/number of eggs) was not significantly different between the two groups (91.5% versus 94.6%), the infected females also had more hatchlings than parasite-free females ($8.6 \pm 0.6 \text{ SE}$ versus $7.6 \pm 0.2 \text{ SE}$; $t = 1.95$, $df = 96$, $p = .05$). Fourteen days after hatching, when nestlings were weighed and maternal blood samples were taken, brood size was not different between the two groups, and there was also no significant difference in the number of fledged young (infected: $6.2 \pm 0.8 \text{ SE}$; uninfected: $6.5 \pm 0.3 \text{ SE}$; $p > .6$). Therefore, a significantly lower percentage of eggs laid by infected mothers led to the production of fledged young (infected: 65.6%; uninfected: 81.5%; $U = 508.5$, $p < .04$).

DISCUSSION

Our analysis shows that females that lay large clutches are most frequently infected with *Plasmodium*, a common blood parasite of many bird species. Positive correlations between reproductive effort and parasite load have also been observed in female bighorn sheep, *Ovis canadensis* (Festa-Bianchet, 1989), in which lactating females had higher fecal counts of lungworm larvae than nonlactating females, and in great tits, where an experimental increase of brood size was correlated

Table 4

Mean clutch size in 1993 and 1994 of individual females in relation to their infection status in the 2 years

Infection status		Clutch size ($\pm \text{SE}$)		n
1993	1994	1993	1994	
-	-	7.6 ± 0.3	7.9 ± 0.4	19
-	+	8.3 ± 0.6	9.8 ± 0.6	7
+	-	9.5 ± 0.6	8.0 ± 1.1	4
+	+	8	9	1

with a higher prevalence of hematozoic parasites (Norris et al., 1994; Richner et al., 1995) in males, but not in females. However, the absence of a correlation has also been reported. In a study on great tits (Allander and Bennett, 1995), there was no correlation between clutch size and the prevalence of blood parasites belonging to various taxa.

While different species or genera of hematozoa may have more or less severe effects on their hosts, there are also good theoretical reasons to expect different outcomes of host-parasite interactions. An outcome predicted by life-history theory (Stearns, 1992) is based on the idea that an individual cannot simultaneously maximize all life-history traits because the energy available to an individual is limited, and an investment in one set of traits can only occur at the expense of a reduced investment in other traits. At the physiological level, there may therefore be a trade-off between energy allocation in reproduction or in a competent immune system (e.g., Gustafsson et al., 1994), which corresponds, at an evolutionary level, to an investment in current versus future reproduction. As a consequence of this trade-off, the individuals that invest more in reproduction will be less resistant to parasites, and a positive correlation between reproductive effort and subsequent parasite load is predicted. In this case, parasitism is a consequence of reproductive investment. Alternatively, the potential of investing in current reproduction may be limited by an already existing parasitemia, and the reproductive effort is therefore a consequence of parasitism. A negative correlation between reproductive effort and parasite load will then be predicted.

Finally, differences between females in their phenotypic quality or quality of the territory may override any reproductive trade-offs. High-quality females, for example, may be able to invest more heavily in both reproduction and immune defense than low-quality females. This will, in a nonmanipulative study, lead to no correlation between reproductive effort and parasite load, as would be predicted by the individual optimization hypothesis (e.g., Boyce and Perrins 1987; Lindén, 1990; Nur 1987; Pettifor et al., 1988). Nonmanipulative studies such as ours, cannot address the question of individual optimization, and a full understanding can only be achieved by an experimental study investigating the trade-off between clutch size and prevalence of pathogens within given phenotype classes.

It has been suggested (Calow, 1979; Stearns, 1992) that the reproductive trade-offs must be mediated by physiological mechanisms. Our findings are interesting in the light of the question concerning the most productive clutch size (Boyce and Perrins, 1987; DeSteven, 1980; Lack, 1966; Nur, 1984; Perrins, 1965; Perrins and Moss, 1975). One of the main explanations for why the most frequent clutch size of many birds is below the most productive clutch size is based on the existence of a trade-off between investment in current and future broods (Charnov and Krebs, 1974), which includes the notion of a cost of reproduction (Williams, 1966). The cost of reproduction is a reduction in either future survival or fecundity. The results of 55 brood manipulation studies (reviewed by Dijkstra et al., 1990; Lindén and Møller, 1989) are equivocal and show that in some cases, but not in others, reproduction affects parental survival and future fecundity.

Higher susceptibility to blood parasites may represent one possible way that a cost is imposed on individuals with a high current investment. Our nonmanipulative study suggests that high reproductive effort is associated with a higher pathogen load, which may represent a major factor concerning the postulated cost of reproduction in the optimal clutch size model. As Stearns (1992: 158) cautions, "all brood size manipulations ignore costs incurred earlier in the life history—in particular the cost of producing eggs." In support of this remark, Tin-

bergen and Dietz (1994), in a nonmanipulative study, showed that natural clutch size is one of the major variables explaining the variation in daily energy expenditure of great tit females tending broods with nestlings 11–12 days of age. Actual brood size at that age did not significantly affect daily energy expenditure. In our study there was a highly significant correlation between prevalence of a blood parasite and clutch size, but no significant correlation with brood size. Our study and the one by Tinbergen and Dietz (1994), however, do not exclude other factors as a cause of the observed relationship. A recent experimental study (Heaney and Monaghan, 1995) found a trade-off between egg production and chick-rearing abilities in common terns (*Sterna hirundo*), and another study documented an intrachlutch trade-off between quantity and quality of eggs produced (Monaghan et al., 1995). Our non-experimental study points to a potential trade-off between the cost of egg production and parasitism, and this relationship is worth evaluating experimentally in the future. Our findings and those mentioned above support previous contentions (Partridge and Harvey, 1985; Stearns, 1992) that the cost of egg production may be important in shaping life-history traits such as clutch size.

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